IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Art Unit	: 1648	**Customer No.: 035811**
Examiner	: Emily M. Le	
Serial No.	: 10/600,361	
Filed	: June 20, 2003	Docket No.: 1187-R-02
Applicants	: Jean-Marie Andrieu	
	: Louis Lu	
Title	: METHODS, AND COMPOSITIONS	Confirmation No.: 7112
	: FOR A THERAPEUTIC ANTIGEN	
	: PRESENTING CELL VACCINE	
	: FOR TREATMENT OF	
	: IMMUNODEFICIENCY VIRUS	

DECLARATION OF MARIE-LISE GOUGEON UNDER 37 C.F.R. 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I hereby declare as follows:

1. I, Marie-Lise GOUGEON, am Head of the Antiviral Immunity, Biotherapy and Vaccine Unit, Institut Pasteur, Paris, France. A copy of my curriculum vitae is attached hereto as Exhibit A.

2. I have read and understood U.S. Patent Application No. 10/600,361 (the "Application"), and have read the Official Action concerning the Application mailed on October 23, 2007.

3. The October 23, 2007 Official Action states that the use of autologous HIV was common in the art at the time of filing and that one skilled in the art would be motivated to use autologous HIV because the virus frequently mutates and would have an expectation of success. I consider one of ordinary skill in the art is to be a person with a PhD in Molecular Biology and

three to five years of laboratory experience working in a field relevant to immunology and HIV pathology.

4. Concerning the alleged obviousness of the claimed subject matter in the Application, it is my understanding that one skilled in the art in view of Belardelli would neither be motivated to modify Belardelli's teachings by use of autologous HIV, nor would he or she have a reasonable expectation of success.

5. For example, at the time that the Application was filed, there were noted difficulties associated with the experimental use of autologous HIV, which would reduce the expectation of successfully modifying Belardelli in the way the Official Action proposes. These difficulties are highlighted by an article published nearly one year after the inventors filed the provisional application underlying the Application. In the article, the authors note that there are technical challenges and difficulties associated with preparation of autologous virus stocks. (See Richman et al. (2003) Rapid evolution of the neutralizing antibody response to HIV-1 type infection. *PNAS*, 100(7): 4144-49; See Discussion, pg. 4149. A copy of the article is enclosed as Exhibit B.)

6. While the Richman et al. study investigated a different aspect of the HIV immune response, the article underscores that use of autologous HIV viruses it not a simple routine procedure. Indeed, in view of Richman et al., one skilled in the art may not reasonably expect that they could even successfully prepare an autologous HIV stock necessary to modify Belardelli in the way that the Official Action proposes. Moreover, if one skilled in the art could not expect to successfully prepare an autologous HIV stock, he or she would be deterred from

attempting to modify the teachings of Belardelli to use autologous HIV and would not reasonably expect success. Accordingly, I do not believe that one of skill in the art in view of Belardelli would be motivated to modify Belardelli by using autologous HIV and arrive at the claimed invention.

7. Furthermore, the lack of a reasonable expectation of successfully modifying Belardelli by using autologous HIV is underscored by the failure of others. For example, such failure can be seen in a post-filing study that also attempted to create a dendritic cell-based vaccine with inactivated autologous HIV. (See Garcia et al. (2005) Therapeutic immunization with dendritic cells loaded with heat-inactivated autologous HIV-1 in patients with chronic HIV-1 infection. J. Infectious Diseases. 191: 1680-85. A copy of the article is enclosed as Exhibit C.)

8. The authors of Garcia et al. attempted to create a vaccine by treating isolated dendritic cells with heat-inactivated autologous HIV, rather than an AT-2-inactivated autologous virus like this Application. The authors indicated that their heat-inactivated vaccine was capable of eliciting "weak and transient" cellular immune responses, and noted that "it could be argued that [their] vaccine did not elicit specific anti-HIV-1 immune responses at all." The authors also specifically note that the observed only a weak virus-specific CD8+ T-cell response, and actually a decrease in the number of circulating CD8+ T-cells. Therefore, in light of the teachings of Garcia et al., one of ordinary skill in the art in view of this article would not reasonably expect that the use of autologous inactivated HIV to create a dendritic cell-based vaccine would be successful in expanding the expression of CD8+ cells.

9. Therefore, based on the experimental results of Garcia et al. and the authors' difficulty in using autologous HIV for the purpose of creating a dendritic cell-based vaccine, I believe that one skilled in the art would have no reasonable expectation of successfully achieving the claimed subject matter by merely modifying Belardelli's methods to use autologous HIV. Indeed, the authors of Garcia et al. suggest even more convincingly that the claimed subject matter is non-obvious when they compare the disappointment of their results to the successes of a study performed by the inventors. Accordingly, I do not believe that the claimed subject matter is obvious in view of Belardelli.

10. The undersigned declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2/19/2008
Date

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EXHIBIT A

INSTITUT PASTEUR

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EDUCATION AND DIPLOMA

1972 : DES Biochemistry, Paris XI University

1974 : Master's degree in Biochemistry, Paris XI University

1975 : Post-graduate Certification in Biochemistry and Microbiology, Institut Pasteur

1978 : PhD in Immunology . Department of Immunology, Institut Pasteur/PVII University

1986 : Doctorate Thesis in Immunology, Paris VII University

POSITIONS AND AWARDS

1975 : Roux Foundation Fellowship

1978 : Research Assistant, Institut Pasteur

1987 : Research Scientist, Institut Pasteur

1987-1990 : Senior Research Scientist, Pediatric Immunology and Rheumatology Unit (INSERM U132), Necker Hospital, Paris

1990-93 Director of the Institut Pasteur Immunology Course, second year of Master

1991-2002 : Immunology Team Leader in Pr. Luc Montagnier' Research Unit, Institut Pasteur

1993-1995 : Associate Professor of Immunology, Paris VII University

Since 1996 : Research Director, Institut Pasteur

Since 2002 : Head of the « Antiviral Immunity, Biotherapy and Vaccine » Unit, Department of Infection and Epidemiology, Institut Pasteur

SCIENTIFIC COMMITEES AND EDITORIAL ACTIVITY

- Member of the scientific committee of Neovacs (2008-)
- Member of the scientific committee ATC Biotherapy, INSERM (2002- 2005)
- Member of the coordinated action of ANRS AC21 on T cell homeostasis and HIV (2001-05)
- Member of several scientific committees in ANRS since 2001
- Member of the scientific committee « HIV and therapy », ABBOTT Company (1999-2005)
- Member of the scientific committee « Lipodystrophy » BMS Company (2000-2004)
- President of the « European Cell Death Organization » (ECDO) 1999-2001
- Associate Editor of *Cell Death and Differentiation* (1997-2003)
- Member of the Editorial Board of *Current Molecular Medicine* (since 2003)
- Member of the Editorial Board of *AIDS* Journal (since 2001)

SCIENTIFIC CONSULTING

- Expert for the Swedish Research Council (Stockholm, Sweden) (2003, 2006, 2008)
- Expert for Messine University, Sicily (2006-2007)
- Consultant for BMS Company (2000-2004, 2008)
- Consultant for ABBOTT Company (1999-2005)
- Expert for EU, 6th PCRDT (2004)
- Consultant for Bayer Company (2002)
- Consultant for Applied Immune Science Company (1993-1995)

DISTINCTIONS

1994: • French Academy of Science/CEA prize for the discovery of Programmed Cell Death in AIDS.

2007 : • Career Award form the European Cell Death Organization

MAIN PUBLICATIONS IN PEER REVIEW JOURNALS

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J. THEZE, L. LECLERCQ, M.L. GOUGEON
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F. POCCIA, CIPRIANI B, VENDETTI S, COLIZZI V, POQUET Y, BATTISTINI L, LOPEZ-BOTET M, FOURNIE J-J, GOUGEON M-L.
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E. LEDRU, LECOEUR H, GARCIA S, DEBORD T, GOUGEON M-L
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J. Immunology (1998), 160: 3194-3206

F. POCCIA, GOUGEON M-L, BONNEVILLE M., LOPEZ-BOTET M, MORETTA A, BATTISTINI L, WALLACE M, COLIZZI, V MALKOVSKY.
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L. WEISS, ROUX A., GARCIA S., DEMOUCHY C., HAEFFNER-CAVAILLON N, KAZATCHKINE M.D., GOUGEON M-L.
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H. LECOEUR, LEDRU E, GOUGEON M-L.
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S. BOULLIER, Y. POQUET, T. DEBORD, J-J FOURNIE, GOUGEON M-L.
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Rapid evolution of the neutralizing antibody response to HIV type 1 infection

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A recombinant virus assay was used to characterize in detail neutralizing antibody responses directed at circulating autologous HIV in plasma. Examining serial plasma specimens in a matrix format, most patients with primary HIV infection rapidly generated significant neutralizing antibody responses to early (0–39 months) autologous viruses, whereas responses to laboratory and heterologous primary strains were often lower and delayed. Plasma virus continually and rapidly evolved to escape neutralization, indicating that neutralizing antibody exerts a level of selective pressure that has been underappreciated based on earlier, less comprehensive characterizations. These data argue that neutralizing antibody responses account for the extensive variation in the envelope gene that is observed in the early months after primary HIV infection.

Neutralizing antibody responses after natural infection or vaccination comprise a major component of protection from virus infection (1). The majority of antibodies directed against the viral envelope glycoprotein (Env) recognizes non-neutralizing epitopes of glycoprotein monomers and is ineffectual (2, 3). Characterizing the neutralizing antibody response to HIV-1 has been limited by technical challenges. The measurement of serial responses to autologous virus has generally required isolation of primary viruses from peripheral blood mononuclear cells, preparation of virus stocks, and titration of these stocks from sequential blood specimens. Neutralizing antibody responses to heterologous primary isolates and to laboratory strains are easier to characterize but seem to develop slowly after infection and to relatively low titers (2, 4, 5).

Neutralization escape mutants of the animal lentiviruses such as equine infectious anemia virus, visna virus, and simian immunodeficiency virus evolve in infected horses, sheep, and rhesus monkeys, respectively (6–8). Neutralizing antibody responses against autologous HIV-1 were reported first by Weiss in 1986 (9), and several later studies have suggested that its appearance is slow to develop and of low titer (2, 4, 5). Neutralization escape of HIV has been reported in limited cases (10–15); however, many studies of autologous neutralizing antibody after primary HIV infection stress the low or absent responses with only infrequent examples of escape (5, 16–18). We report here that in most patients, potent neutralizing antibody responses are generated early after infection, at first to the autologous infecting HIV variant and then to subsequent variants. The antibody responses to these variants exert a selective pressure that drives continuous evolution of neutralization escape mutants.

Materials and Methods

Study Subjects. Study subjects were recruited with a diagnosis of primary (recent) HIV infection as part of the San Diego Acute and Early Infectious Disease Research Program. Serial blood specimens were collected, separated by centrifugation into plasma and cells, and frozen at −70°C. All subjects signed informed consents to protocols approved by the University of California Human Subjects Committee (La Jolla).

Neutralization Assay. A recombinant virus assay initially developed to measure antiretroviral drug resistance during a single round of virus replication was adapted to measure virus-antibody neutralization (19). HIV genomic RNA was isolated from virus stocks or plasma by using oligo(dT) magnetic beads. First-strand cDNA was synthesized in a standard reverse transcription reaction by using an oligo(dT) primer. Env DNA (gp160) was amplified by PCR using forward and reverse primers located immediately upstream and downstream of the env initiation and termination codons, respectively. The forward and reverse primers contain recognition sites for *Pin*AI and *Mlu*I, respectively. Env PCR products were digested with *Pin*AI and *Mlu*I and ligated to compatible ends in the pCXAS expression vector, which uses the cytomegalovirus immediate-early promoter enhancer to drive env insert expression in transfected cells (Fig. 1*A*). Ligation products were introduced into competent *Escherichia coli* (Invitrogen) by transformation, and pCXAS-env plasmid DNA was purified from bacterial cultures (Qiagen, Valencia, CA). An aliquot of each transformation was plated onto agar, and colony counts were used to estimate the number of envelope sequences represented in each pCXAS-env library (generally 500–5,000 clones). Sequence analysis of individual pCXAS-env clones (10–20) was used to verify the heterogeneous composition (i.e., quasispecies) of pCXAS-env libraries. Virus particles containing patient virus envelope proteins were produced by cotransfecting HEK293 cells with pCXAS-env libraries plus an HIV genomic vector that contains a firefly luciferase indicator gene (Fig. 1*A*). pCXAS-env plasmid preparation and HEK293 cell-transfection conditions have been optimized to ensure consistent virus particle production. Recombinant viruses pseudotyped with patient virus envelope proteins were harvested 48 h posttransfection and incubated for 1 h at 37°C with serial 4-fold dilutions of heat-inactivated patient plasma samples (antibody) (Fig. 1*B*). U87 cells that express CD4 plus the CCR5 and CXCR4 coreceptors were inoculated with virus-plasma (antibody) dilutions in the absence of added cations. Virus infectivity was determined 72 h postinoculation by measuring the amount of luciferase activity expressed in infected cells. Neutralizing activity is displayed as the percent inhibition of viral replication (luciferase activity) at each antibody dilution compared with an antibody-negative control: % inhibition = (1 − [luciferase + Ab/luciferase − Ab]) × 100. Titers were calculated as the reciprocal of the plasma dilution conferring 50% inhibition (IC_{50}), which is demarcated as a dashed vertical line in Fig. 2. A series of experiments using diluted virus stocks (1:2, 1:5, 1:10, or 1:20) has demonstrated that luciferase activity correlates with virus inoculum, but that antibody neutralization titers are not significantly affected (data not shown).

Results

Measuring the Autologous Neutralizing Antibody Response. We began our investigation by studying 14 subjects who presented to the

Abbreviation: Env, viral envelope glycoprotein.

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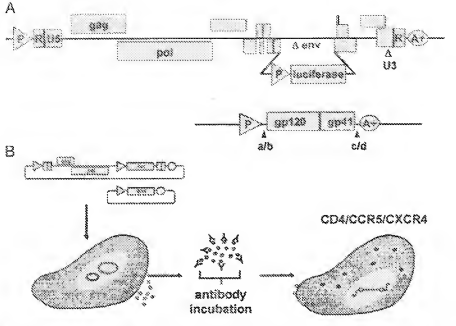

Fig. 1. (*A*) Diagrams of the expression vectors used to generate the pseudovirions used in the neutralization assay. The envelope-defective, luciferase-expressing vector is above the vector that expresses the full-length envelopes amplified from patient plasmas. (*B*) Schema of the generation of pseudovirions by cotransfection of the two vectors depicted in *A*. These pseudovirions then are incubated for 1 h with serial 4-fold dilutions of plasma or antibody solutions before infection of the U87-derived target cells to generate luciferase activity.

San Diego Acute and Early Infection Disease Research Program 30–65 days after their estimated date of HIV infection and elected to defer or delay antiretroviral therapy. Plasma samples (3–13 per patient) were obtained at presentation to the clinic and at regular intervals for 6–39 months of follow-up. Neutralization activity was measured by using a cell-based infectivity assay that greatly facilitates the characterization of antibodies and virus envelope proteins derived from the same plasma sample (i.e., autologous envelope–antibody pairs). Infectivity is measured by using recombinant viruses that carry a luciferase reporter gene and are pseudotyped

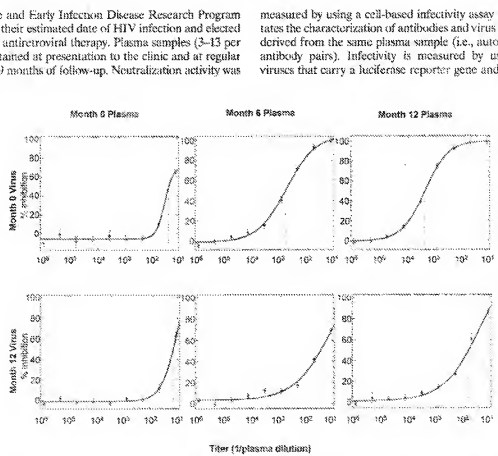

Fig. 2. Neutralization of autologous HIV. The neutralizing activity of plasmas obtained from patient 1 at months 0, 6, and 12 after presentation with primary infection is assayed against virus from months 0 and 12. The titer is defined as the reciprocal of the dilution of plasma that produces 50% inhibition of virus replication (dashed lines). The error at each dilution reflects the standard error of duplicate wells.

Table 1. Antibody neutralization titers (subject TN-1, treatment naive)

Virus, months	Plasma, months								
	0	3	6	9	12	15	18	21	25
0	26	219	675	1403	2670	2089	2190	2369	2411
3	29	179	1024	2151	3733	3152	2808	2953	3086
6	27	35	78	358	1769	1939	2247	3112	4345
9	36	67	82	200	795	1078	1371	2208	3375
12	19	48	36	64	76	166	556	937	1407
15	29	43	64	76	90	119	374	721	1234
18	42	65	61	152	117	134	122	289	526
21	41	66	82	84	85	113	78	107	296
25	42	62	56	62	85	77	55	61	95
Controls									
NL43	17	138	294	956	1172	953	1584	1868	2143
JRCSF	24	37	35	60	87	97	105	152	209
AMPHO	<10	32	14	13	14	13	<10	<10	31

Neutralizing HIV antibody titers of sequential plasma specimens against autologous virus. Serial plasmas were obtained from three untreated patients presenting with primary HIV infection. The titer of each plasma against its concurrent virus specimen is in bold type. Control viruses include an amphotropic murine leukemia virus (AMPHO), a neutralization-sensitive X4-tropic virus (NL4-3), and a relatively neutralization-resistant R5-tropic virus (JR-CSF).

with patient HIV envelope proteins (Fig. 1). Fig. 2 demonstrates the ability of this assay to detect the emergence of autologous neutralization activity directed against the virus present at presentation of primary HIV infection (month 0) in serial plasma samples (0, 6, and 12 months).

This assay consistently generates neutralization curves similar in shape and slope and with little variability in duplicate assay wells (Fig. 2). As a result, IC_{50} titers (1/dilution that confers 50% neutralization) were typically 5-fold higher than the IC_{80} values and 10-fold higher than the IC_{90} values. The IC_{50} titers are reported because they can be most precisely derived from the linear portion of the sigmoid curve. In contrast to most published assays, plasmas with IC_{50} titers >100 in this assay have less than a 1% nonneutralized fraction (i.e., inhibition curves typically plateau at 100% neutralization). To monitor the amount of neutralization activity that is not mediated by antibodies directed against HIV-1 env proteins, each plasma sample was also tested

Table 2. Antibody neutralization titers (subject TN-2, treatment naive)

Virus, months	Plasma, months									
	0	2	5	10	17	20	24	27	29	32	36
0	51	53	53	72	56	87	80	66	69	76	57
2	45	48	46	62	59	77	65	56	54	64	63
5	46	51	42	57	38	54	52	43	49	60	55
10	52	57	37	58	50	73	81	67	58	59	46
17	44	41	<10	61	38	61	55	70	83	64	41
20	62	50	<10	119	69	86	94	122	75	104	54
24	66	79	66	78	59	115	166	78	88	100	72
27	50	96	49	101	56	84	95	97	61	116	82
29	71	63	<10	114	59	88	80	56	61	111	53
32	65	48	159	118	53	72	70	67	46	44	44
36	51	83	<10	85	59	116	82	93	75	40	NT
Controls											
NL43	46	69	90	129	123	212	221	181	172	138	207
JRCSF	34	39	28	39	31	39	44	32	31	28	30
AMPHO	<10	25	16	28	17	NT	32	NT	22	20	33

See Table 1 legend for details.

Table 3. Antibody neutralization titers (subject TN-3, treatment naive)

Virus, months	Plasma, months									
	0	3	6	10	14	19	22	30	39	39
0	39	67	103	102	152	303	376	403	362	449
3	47	69	142	231	261	547	488	419	392	464
6	37	50	81	91	172	340	308	360	386	363
10	32	34	47	75	117	295	321	336	400	406
14	34	43	50	45	69	164	147	235	236	245
19	29	39	54	51	50	67	62	188	235	223
22	37	37	45	51	44	41	55	185	311	221
30	24	29	43	48	34	33	79	44	56	90
35	27	30	34	32	29	31	29	41	33	41
39	40	36	53	59	40	49	27	45	36	40
Controls										
NL43	29	63	104	197	261	733	509	610	662	744
JRCSF	23	23	28	26	32	75	65	72	67	70
AMPHO	35	23	27	29	NA	39	49	45	45	20

See Table 1 legend for details.

against a recombinant virus stock that was pseudotyped with amphotropic murine leukemia virus envelope proteins (gp70SU and p15TM). Typically, the IC_{50} values of amphotropic murine leukemia virus controls were <50.

Autologous Neutralizing Antibody Response in Patients with Primary HIV Infection. The neutralization activities of sequential plasma samples against sequential virus envelope proteins from the same patient (autologous responses) or against two reference viruses (heterologous responses) are displayed in Tables 1–3. For 6 of the 14 patients, peak neutralizing antibody titers reached >1,000 as exemplified in patient TN-1. For two patients, negligible neutralizing antibody titers (<100) to autologous viruses were generated as exemplified in patient TN-2. For the remaining six patients, peak titers to autologous virus ranged between 100 and 1,000 as exemplified in patient TN-3; however, for three of these patients the period of follow-up was <12 months, and antibody neutralization titers may not have peaked yet.

Time of Appearance of the Autologous Antibody Response. To address more precisely the time of appearance of measurable neutralizing antibody responses, more frequent serial plasmas were examined from three patients shortly after the onset of symptoms of primary HIV infection. In patient TN-1 for example (Table 4), neutralizing activity could be discerned 4–8 weeks

Table 4. Initial detection of antibody neutralization activity for subject TN-1

Virus, week	Plasma, week					
	0	2	3	4	8	12
0	36	38	42	58	184	319
2	41	43	37	54	200	437
3	26	42	38	55	236	490
4	40	50	52	68	277	518
8	30	46	49	64	246	465
12	36	45	37	59	183	296
AMPHO	22	20	<15	15	26	19

The time course of development of neutralizing antibody in frequently obtained plasmas from patient 1 early after infection is shown. Sequential plasmas were obtained at the indicated weeks after presentation against sequential autologous viruses. The values of concurrent assays are in bold type. AMPHO, amphotropic murine leukemia virus.

Table 5. Fifty percent neutralization titers (μg/ml) by monoclonal antibodies

Patient no.	Virus, month	b12	2F5	2G12
TN-1	0	2.3	10.5	**>50**
	3	3.1	10.2	**>50**
	6	2.4	3.7	1.2
	9	0.4	2.1	2.1
	12	2.4	3.1	1.3
	15	3.7	2.5	0.8
	18	6.6	1.8	0.4
	21	**>25**	2.9	0.9
	25	**>25**	4.7	4.3
TN-2	0	**>25**	1.9	8.0
	3	**>25**	1.8	9.6
	9	**>25**	1.9	8.2
	15	**>25**	1.5	5.7
	23	**>25**	2.1	4.4
	28	**>25**	2.4	3.8
	35	**>25**	2.7	6.2
TN-3	0	12.1	9.4	1.5
	7	**>25**	4.8	0.7
	15	**>25**	3.7	0.5
	24	**>25**	7.0	**>50**
	37	**>25**	13.4	**>50**
	41	**>25**	12.1	**>50**
TN-4	0	**>25**	19.0	**>50**
	3	**>25**	17.9	**>50**
	6	**>25**	9.4	**>50**

Susceptibility of sequential virus isolates from patients 1–4 to neutralization by three broadly reactive monoclonal antibodies is shown. The values are the concentration of antibody (in μg/ml) that produces 50% inhibition of virus replication.

after presentation, characteristic of those patients with neutralizing antibody responses. The neutralizing responses to a heterologous primary isolate (JR-CSF) and laboratory strain (NL4-3) were delayed and of modest magnitude consistent with the published literature (2, 4, 5). The detection of this initial response required a sensitive and accurate assay using early autologous virus and antibody. The true timing of emerging neutralizing antibody responses may be masked by the extensive levels of virus replication ($\approx 10^{10}$ virions generated daily during chronic infection (20) and 100 times that during acute infection (21). Therefore, much of the neutralizing antibody that is generated early in infection may be bound to virions in lymphoid germinal centers and elsewhere and thus undetectable in plasma.

Investigation of Poor Autologous Neutralizing Antibody Responses. The failure of 2 of 14 patients to generate a significant neutralizing antibody response (Table 2) and the varying levels and timing of peak antibody titers among the untreated patients did not seem to correlate with levels of plasma HIV RNA or CD4 lymphocyte counts during the period of follow-up (data not shown). To address whether a generalized or inherent neutralization susceptibility of the patients' viruses accounted for this variability, viruses derived from two subjects who did not generate neutralization responses (TN-2 and TN-4) and two subjects who did generate neutralization responses (TN-1 and TN-3) were tested against three well characterized, broadly neutralizing monoclonal antibodies (b12, 2F5, and 2G12) (Table 5; refs. 22–24). Monoclonal antibody neutralization patterns did not correlate with the presence or absence of an autologous neutralizing antibody response. Viruses derived from all time points from each subject were susceptible to at least one monoclonal antibody. Thus viruses are not inherently resistant to neutralization. Notably, for subject TN-1 the appearance of a 2G12 neutralization-sensitive virus at month 6 and the disappearance of an IgG1b12 neutralization-sensitive virus at month 21 exemplifies the continual evolution of virus envelope sequence in response to neutralizing antibody. In contrast, the two patients who failed to develop measurable neutralizing antibody responses did not evolve changes in response to these monoclonal antibodies. Preliminary sequencing analysis suggests that neutralization escape involves multiple variations throughout *env* that included missense mutations, insertions, deletions, and glycosylation site mutations, often as mixtures of clones or in combinations on clones (data not shown). This complexity of *env* sequence evolution defies a single simple explanation for evolution of neutralization escape between time points.

Table 7. Antibody neutralization titers for Subject TE-1 (treatment experienced)

	Plasma, months							
Virus, months	0	2	5	8	11	14	17	19
0	**107**	193	292	264	505	504	519	440
2	113	62	160	191	370	435	475	335
5	85	52	**119**	165	255	248	388	279
Controls								
NL43	76	108	153	149	145	85	134	99
JRCSF	88	57	134	166	155	100	152	71
AMPHO	59	34	90	130	140	106	113	57

The neutralizing antibody titers are depicted over time against three viruses that could be tested before plasma virus became undetectable. AMPHO, amphotropic murine leukemia virus. The values of concurrent assays are in bold type.

Crossreactivity of Neutralizing Responses to Heterologous Viruses. To address further whether the observed variability in neutraliza-

Table 6. Antibody neutralization titers against heterologous viruses

	Plasma																	
	TN-1, month			TN-2, month			TN-3, month			TN-6, month			TN-7, month			TN-9, month		
Virus, month 0	0	6	12	0	7	11	0	6	11	0	6	12	0	6	12	0	6	12
TN-1	**54**	1236	3677	70	66	52	34	38	40	35	45	79	41	40	109	83	40	27
TN-2	27	42	67	**44**	**78**	**73**	17	<15	21	44	22	30	22	27	89	66	32	28
TN-3	<15	22	36	37	25	22	**54**	**3020**	**1435**	<15	16	23	<15	<15	33	37	<15	<15
TN-6	45	56	59	44	53	49	20	27	26	**62**	**355**	**1097**	28	47	126	99	51	33
TN-7	47	55	67	57	70	54	25	23	33	39	54	81	**41**	**2915**	**3741**	90	53	51
TN-9	50	48	43	62	71	60	41	36	30	39	66	72	23	24	91	70	**374**	**991**
AMPHO	20	22	19	43	29	22	<15	<15	<15	17	22	23	16	80	85	<15	<15	<15

Cross neutralization among plasmas and viruses from patients with primary HIV infection. The month-0 viruses from 13 patients were assayed for neutralization activity against serial plasmas from 13 patients, of which six representative results are displayed. The autologous reactions are in bold type. AMPHO, amphotropic murine leukemia virus

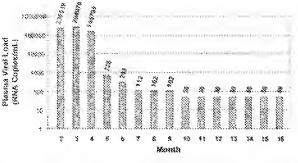

Fig. 3. Plasma HIV viral load in patient TE-1, who initiated potent antiretroviral therapy 16 weeks after presentation (see Table 7). The plasma HIV RNA values over time are shown

tion responses was attributable to variability in antibody response or in virus susceptibility to neutralization, cross-neutralization assays were performed with 13 of the month-0 isolates and several plasma specimens from each of the corresponding patients (Table 6). Compared with autologous viruses, neutralization of heterologous viruses was absent or at best negligible during the first year of HIV infection. The possibility that plasma samples from patients with poor neutralizing responses contained blocking antibodies or other inhibitors of neutralization was investigated by mixing plasma samples from the two patients with poor responses with neutralization-positive plasmas to look for reduced titers against neutralization-sensitive viruses. No suggestion of such interference was observed (data not shown).

Impact of Potent Antiretroviral Therapy of Neutralizing Antibody Responses. Using a second group of subjects with recent HIV infection, we investigated the impact of the administration of potent antiretroviral therapy on the neutralizing antibody response. To conduct these studies, a genomic HIV vector was constructed by using a *pol* gene derived from a patient virus that was highly resistant to protease and reverse-transcriptase inhibitors. This vector, in conjunction with patient virus envelope expression vectors can be used to measure neutralizing antibody accurately despite the presence of inhibitory drugs in plasma of treated patients that confound standard neutralization assays (data not shown). Autologous antibody neutralization activities were measured in longitudinal plasma samples collected from five patients who were administered antiretroviral drugs shortly after presentation and sustained suppression of plasma HIV RNA below 50 copies per ml. In all five subjects, antibody titers plateaued at relatively low titers (<1:500), and their spectrum of activity evolved very little. This pattern is exemplified by patient TE-1 (Table 7 and Fig. 3).

Individual Variability of Neutralizing Antibody Responses. The impact of antiretroviral treatment on the emergence and evolution of neutralization responses can be appreciated by comparing the patterns of individual responses among seven patients who declined treatment and five patients who successfully suppressed plasma HIV RNA with antiretroviral therapy (Fig. 4). Fig. 4 also depicts

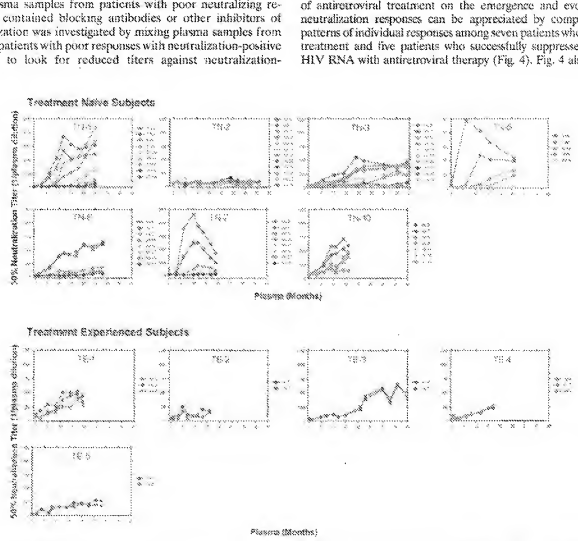

Fig. 4. Variable individual autologous neutralizing responses. The autologous neutralizing antibody responses are displayed for seven primary HIV infection patients who declined antiretroviral therapy and for five patients who initiated potent suppressive therapy within 3 months of seroconversion.

the considerable intersubject variation in the time to peak titer and the potency of neutralizing antibody responses directed at viruses that emerged later in infection. In 9 of the 12 untreated patients with detectable neutralizing antibody, the highest measured neutralization titer was directed against the baseline virus (month 0) whereas in three others higher titers of neutralizing antibody developed against viruses that emerged later in infection.

Discussion

The role of neutralizing antibody in modulating the natural course of infection or as a vaccine strategy has received limited attention for several reasons. Neutralizing antibody responses, especially to autologous viruses, have been difficult to measure because of the technical challenges associated with the preparation of autologous virus stocks that are typically obtained from peripheral blood mononuclear cells. Furthermore, cell-derived virus does not accurately reflect the actively replicating population present in plasma; the detection of drug-resistance mutations in lymphocytes lags >1 month behind those detectable in plasma virus (25–27). To date, immunizations with envelope proteins (or expression vectors) have proved disappointing, generating low levels of neutralizing antibody or antibody restricted to the autologous strain and laboratory-adapted strains but lacking activity against most primary isolates (2, 3). In addition, the interest in neutralizing antibody has also been overshadowed by studies that implicate cell-mediated immunity in the control of HIV/simian immunodeficiency virus infection. Partial control of HIV replication *in vivo* has been temporally associated with the appearance of cytotoxic CD8 T cell responses (28). In simian immunodeficiency virus infection, the elimination of CD8 lymphocytes significantly releases simian immunodeficiency virus replication from partial immune control (29, 30).

The rate of antibody neutralization escape and evolution in recently infected, untreated patients described in this report exceeds the relatively rapid rates of change that are characteristic of the emergence of drug resistance during suboptimal antiretroviral therapy. This observation indicates that the potency of the selective pressure exerted by neutralizing antibodies can account for the extensive variability of *env* in comparison to other HIV genes (31). The question then arises why such a strong selective pressure fails to appreciably impact levels of virus replication as does chemotherapy. During the course of HIV evolution, the envelope protein has acquired the ability to retain function (i.e., bind receptors) while tolerating multiple and repeated changes in several highly variable regions containing numerous glycosylation sites (32). Although drug-resistance mutations confer much greater fitness in the presence of antiretroviral drugs, they typically do not exist as common polymorphisms in untreated patients because they impair the replication of wild-type viruses. In contrast, during the natural course of early HIV infection, fully functional envelope variants continuously emerge and compete for outgrowth in the presence of a rapidly evolving neutralizing antibody response.

The lack of cross-neutralizing antibody responses against heterologous primary isolates during the early stages of HIV infection adds to existing concerns about the difficulty of identifying immunogens capable of inducing broadly protective responses. It will be of interest to determine whether more broadly reactive antibody responses evolve over a longer course of HIV infection (i.e., >39 months). Nevertheless, an optimist might argue that neutralizing antibody confers such potent selective pressure that antibody targeted against a broad range of circulating viruses could contribute to an effective HIV vaccine. Moreover, in contrast to the selection for escape by a narrowly focused, potent neutralizing response that is reactive to remarkably high levels of virus replication, the prophylactic use of such potent activity against a relatively modest inoculum might confer significant levels of protection and is consistent with the efficacy of passive prophylaxis with antibody to autologous virus in the macaque model (33–37).

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EXHIBIT C

BRIEF REPORT

Therapeutic Immunization with Dendritic Cells Loaded with Heat-Inactivated Autologous HIV-1 in Patients with Chronic HIV-1 Infection

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Therapeutic immunization with autologous monocyte-derived dendritic cells (DCs) loaded with heat-inactivated autologous human immunodeficiency virus type 1 (HIV-1) in 12 patients with chronic HIV-1 infection who were receiving highly active antiretroviral therapy (HAART) was feasible, safe, and well tolerated. Virus was obtained during an initial interruption of HAART (hereafter, "stop 1") so that DCs could be pulsed. After immunization and a second interruption of HAART (hereafter, "stop 2"), set-point plasma viral load (PVL; 24 weeks after stop 2) decreased ≥0.5 log_{10} copies/mL relative to baseline PVL in 4 of 12 patients. We observed a significant lengthening in mean doubling time of PVL rebound and significant decreases in the area under the curve and the mean peak of PVL rebound after stop 2, compared with those after stop 1. This response was associated with changes in HIV-1–specific $CD4^+$ lymphoproliferative and $CD8^+$ T cell responses. These changes were not observed in a group of nonimmunized control patients.

It has been suggested that ex vivo–generated dendritic cells (DCs) might be the most potent cellular adjuvant for a therapeutic HIV-1 vaccine. Although HIV-1 infection can adversely affect DC function, monocyte-derived DCs (MD-DCs) isolated from patients with HIV-1 infection and grown in granulocyte-macrophage colony-stimulating factor and interleukin-4 for 1 week were mostly uninfected and functionally intact [1, 2]. Mature DCs isolated from chronically infected individuals and infected with canarypox virus elicited strong anti-HIV $CD8^+$ and $CD4^+$ T cell responses in vitro [3], and intravenous infusion of allogeneic DCs pulsed with recombinant HIV-1_{MN} gp160 or synthetic peptides in HIV-1–infected patients was safe and enhanced the immune response to HIV-1 (although it was unable to control viral replication) [4]. It was recently reported that therapeutic immunization with autologous DCs in antiretroviral-naive patients chronically infected with HIV-1 elicited effective cellular immune responses [5]. In the present study, we assessed the safety of and the virological and HIV-1–specific immune responses after therapeutic vaccination with autologous MD-DCs loaded with autologous heat-inactivated HIV-1 in patients with nonadvanced chronic HIV-1 infection who were receiving highly active antiretroviral therapy (HAART).

Patients, materials, and methods. Eighteen patients from the SCAN study [6] with nonadvanced chronic HIV-1 infection who had baseline and nadir $CD4^+$ T cell counts of >500 cells/μL and baseline plasma viral loads (PVLs) of >5000 copies/mL before receipt of any HAART and with PVLs of <20 copies/mL for at least 104 weeks while receiving HAART were randomized (2:1) either to be immunized with autologous MD-DCs pulsed ex vivo with whole autologous heat-inactivated HIV-1 (hereafter, "DC vaccine") ($n = 12$) or to be a control patient ($n = 6$). Seventy-eight weeks before the first immunization, HAART was interrupted (hereafter, "stop 1"); when PVL peaked, 3 plasmaphereses were performed, to obtain autologous virus so that DCs could be pulsed. We did not find any specific patient characteristic that predicted the level of PVL rebound after stop 1. Thereafter, HAART was reinitiated, and PVLs decreased to <20 copies/mL in all patients within 12 weeks. After 78 weeks, 5 immunizations were performed at intervals of 6 weeks. One week before each immunization, plasma monocytes were obtained and cultured for 8 days under

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clinical-grade good manufacturing practice conditions, to develop MD-DCs as described elsewhere [3]. After virus inactivation for 30 min at 56°C, these DCs (median, 2×10^6 cells) were pulsed with the autologous virus 24 h before being injected subcutaneously (mean, 5×10^6 virions/immunization; see table 1). The first immunization was a negative control (mock) immunization with MD-DCs not pulsed with HIV-1. Six weeks after the last dose of DC vaccine was administered (week 30), HAART was interrupted again (hereafter, "stop 2"), and the patients were followed for at least 24 weeks. In previous in vitro experiments, we assessed the adequate maturation of MD-DCs (data not shown) and found that MD-DCs from patients with nonadvanced chronic HIV-1 infection who were receiving HAART, when loaded with a heat-inactivated whole laboratory strain of HIV-1 or recombinant HIV-1 proteins, were able to strongly induce the activation of autologous $CD4^+$ and $CD8^+$ T cells (data not shown).

The main end points of the present study were tolerance and the proportion of patients with a set-point PVL decrease of ≥ 0.5 $\log_{10}$ copies/mL 24 weeks after stop 2 (week 54), relative to the baseline PVL (before receipt of any HAART). The patients' baseline PVLs and $CD4^+$ cell counts (table 1) were calculated as the median of all measurements (median, 6 measurements; range, 3–8 measurements) available for the 2 years preceding the initiation of any HAART. Secondary end points were the dynamics of PVL rebound after the immunizations with DC vaccine and stop 2, compared with those after stop 1; HIV-1–specific immune responses (Th1 cell levels, cytotoxic T lymphocyte [CTL] levels, and serum neutralizing-antibody titers); and changes in lymphoid tissue, which were assessed as described elsewhere [7–9]. Tonsillar biopsies were performed in immunized patients who had accessible tonsillar tissue (8/12) at week 0 (before the initial mock immunization with nonpulsed DCs) and week 30 (6 weeks after the last dose of DC vaccine was administered); no biopsies were performed in control patients. All patients provided written, informed consent, and the present study was approved by the institutional ethics review board.

Quantitative data were compared between groups by use of the Wilcoxon matched-pairs test. Changes in PVL over a period of 12 weeks after stop 1 and stop 2 were analyzed by use of an area-under-the-curve (AUC) measurement. Spearman rank order correlations were performed on quantitative data. $P < .05$ was considered to be statistically significant.

Results. The baseline characteristics of the patients are shown in table 1. There were no clinically important or statistically significant differences between the immunized patients and the control patients, except in baseline PVL, which was lower in the control patients. Two control patients left the study, 1 because of relocation and 1 because of a diagnosis of lung cancer. Overall, the tolerance of the DC vaccine was good. There were no local reactions. Two of the 12 immunized patients developed mild flu-like reactions 24 h after immunization. Injections of the DC vaccine were not associated with any clinical or serologic evidence of autoimmunity in the patients (data not shown).

There was no significant change in mean PVL between baseline (before receipt of any HAART) and the set point reached 24 weeks after stop 2 ($P = .53$) (figure 1 and table 1). However, there was a decrease in set-point PVL of ≥ 0.5 $\log_{10}$ (defined as a "virological response") in 4 of the 12 immunized patients (change, −0.94, −0.68, −0.72, and −0.67 $\log_{10}$ copies/mL in patients 1, 2, 3, and 4, respectively). No virological responses were observed in the control patients (table 1). There was no significant difference in the proportion of patients with a virological response between the immunized patients and the control patients ($P = .51$). In lymphoid tissue, the mean ± SE tonsillar tissue viral load decreased from 3.35 ± 0.42 $\log_{10}$ copies/mg of tissue at week 0 to 2.76 ± 0.5 $\log_{10}$ copies/mg of tissue at week 30 ($P = .10$).

The dynamics of PVL rebound during the first 12 weeks after stop 1 and stop 2 were also evaluated and compared between groups. We observed a significant lengthening in mean doubling time of PVL rebound ($P = .01$) and significant decreases in the AUC ($P = .02$) and the mean peak of PVL rebound ($P = .004$) after stop 2, compared with those after stop 1 (table 1). No virological changes in the dynamics of PVL rebound occurred in the control patients.

The serum neutralizing-antibody titers did not change significantly after the series of immunizations (data not shown). Both the magnitude and the breadth of the total HIV-1–specific $CD8^+$ T cell responses (defined as the sum of individual responses per patient) decreased progressively during the series of immunizations. The median frequencies of the total HIV-1–specific $CD8^+$ T cell responses at week 0 and after vaccination at weeks 6, 12, 18, 24, and 30 were 1347, 1482, 1436, 948, 548, and 504 spot-forming cells/1×10^6 peripheral-blood mononuclear cells, respectively ($P = .0008$). The breadth of the total HIV-1–specific $CD8^+$ T cell responses (defined as the number of peptides recognized per patient) decreased from a median of 6 peptides (range, 1–17 peptides) at week 0 to a median of 2 peptides (range, 0–9 peptides) at week 30 ($P = .0008$). When pools of overlapping peptides (p24, p17, and p2p7p1p6 proteins) were tested, a similar pattern of changes in the median frequency of total HIV-1 Gag-specific T cell responses were observed during the series of immunizations (data not shown). $CD8^+$ T cell responses recovered progressively after stop 2. No changes in $CD8^+$ T cell responses were observed in the control patients. The decrease in the magnitude of the $CD8^+$ T cell responses in the patients with a virological response (hereafter, "the responders") was similar to that in the patients without a virological response (hereafter, "the nonresponders"). Conversely, the greater the decrease in the magnitude of the total

Table 1. Characteristics of immunized and control patients, changes in plasma viral load (PVL), and quantity of HIV-1 (obtained by plasmapheresis of 1800 mL of plasma from immunized patients) that was used for loading dendritic cells (DCs).

Category, patient (sex, age in years)	Baseline $CD4^+$ T cell count, cells/mL[a]	HIV-1 RNA for pulsing DCs, log_{10} copies	PVL, log_{10} copies/mL		PVL rebound					
					Doubling time, days		AUC[b]		Peak, log_{10} copies/mL	
			Baseline[a]	Week 54	After stop 1	After stop 2	After stop 1	After stop 2	After stop 1	After stop 2
Immunized patients										
1 (M, 29)	793	7.19	4.24	3.30	1.89	2.23	3.39	2.52	5.16	5.30
2 (M, 45)	707	7.90	4.58	3.90	1.76	3.95	3.28	1.40	5.07	3.09
3 (M, 56)	614	8.22	4.51	3.79	2.67	3.36	3.67	2.61	5.75	4.56
4 (F, 32)	628	8.20	5.16	4.43	1.78	2.59	3.47	3.07	5.00	4.55
5 (M, 30)	781	7.07	4.12	4.12	2.23	2.60	2.29	3.20	4.84	4.46
6 (M, 54)	588	7.42	3.94	3.92	1.79	3.46	2.82	2.40	5.40	4.51
7 (M, 37)	1005	7.63	4.15	4.67	1.96	5.02	3.16	2.16	4.89	4.93
8 (M, 48)	763	7.56	4.65	4.80	1.37	1.83	3.48	2.96	6.37	4.92
9 (M, 47)	764	7.14	4.02	4.03	1.77	2.73	3.27	3.04	5.35	4.67
10 (M, 26)	759	9.11	5.22	6.03	1.82	1.59	4.03	3.93	6.15	5.67
11 (M, 31)	948	7.49	4.60	4.63	1.64	1.78	3.55	3.11	5.70	5.42
12 (M, 46)	706	7.96	4.11	4.70	2.76	2.30	3.32	3.36	5.14	4.96
Mean	754	7.74	4.44	4.27	1.95	2.82	3.32	2.87	5.41	4.75
SE	36	0.16	0.12	0.15	0.12	0.28	0.13	0.19	0.14	0.18
Control patients										
1 (F, 43)	879	...	3.07	3.01	6.51	5.14	1.93	1.78	3.86	4.23
2 (M, 58)	619	...	3.74	3.43	5.55	4.95	1.67	1.56	3.89	4.08
3 (M, 35)	900	...	3.64	3.36	4.70	4.08	2.15	2.61	3.96	3.80
4 (M, 39)	504	...	3.73	3.54	2.70	2.56	2.48	3.33	5.19	5.09
Mean	725	...	3.54	3.34	4.86	4.18	2.06	2.31	4.22	4.30
SE	97	...	0.16	0.11	0.81	0.56	0.17	0.41	0.32	0.28

NOTE. Boldface indicates those 4 patients with a PVL decrease of ≥ 0.5 log_{10} copies/mL between baseline (before receipt of any highly active antiretroviral therapy [HAART]) and week 54 (after immunization and 24 weeks after the second interruption of HAART [stop 2]); these patients were defined as having a virological response. AUC, area under the curve; stop 1, first interruption of HAART.

[a] Median of all measurements available for the 2 years preceding the initiation of any HAART.

[b] Calculated with data from the first 12 weeks after stop 1 and stop 2.

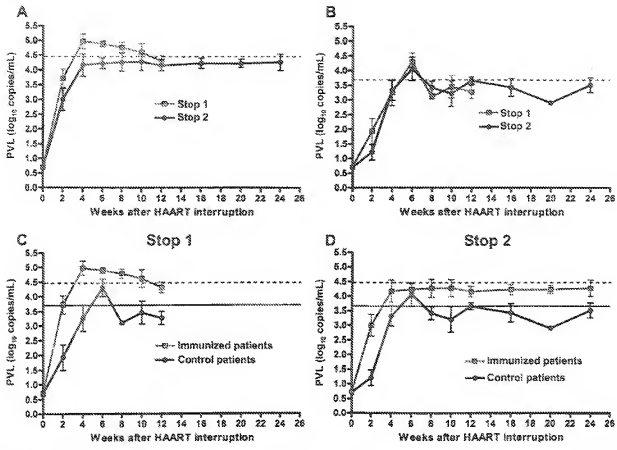

Figure 1. Plasma viral load (PVL) rebound after the first (stop 1) and second (stop 2) interruptions of highly active antiretroviral therapy (HAART). The dashed and continuous lines represent the mean baseline PVLs (before receipt of any HAART) of immunized patients (who were immunized between stops 1 and 2) and control patients, respectively. *A*, Mean PVLs of immunized patients. *B*, Mean PVLs of control patients. *C*, Mean PVLs of immunized patients and control patients after stop 1. *D*, Mean PVLs of immunized patients and control patients after stop 2.

$CD8^+$ T cell responses, the greater the lengthening of the doubling time of PVL rebound from stop 1 to stop 2 ($r = -0.77$; $P = .004$). In lymphoid tissue, we found a significant increase in total CTL level ($CD8^+$ and granzyme B^+ cells) in the intrafollicular area, from a mean ± SE of 7.2 ± 1.9 cells/high-power field (HPF) at week 0 to a mean ± SE of 12.5 ± 3.8 cells/HPF at week 30 ($P = .05$). The increase in total CTL level was directly correlated with the lengthening of the doubling time of PVL rebound from stop 1 to stop 2 ($r = 0.85$; $P = .03$).

HIV-1–specific $CD4^+$ lymphoproliferative response (LPR) to p24 antigen increased slightly and nonsignificantly after the first 2 doses of DC vaccine were administered (analyzed at weeks 12 and 18). The median total stimulation indices at week 0 and after vaccination at weeks 6, 12, 18, 24, and 30 were 2.66, 2.18, 3.75, 4.73, 2.89, and 1.88, respectively ($P = .26$). The responders had a weak but significant increase in LPR during the series of immunizations, compared with that in the nonresponders (change in stimulation index, mean ± SE of 3.09 ± 1.7 and −0.21 ± 0.44, respectively) ($P = .03$). The increase in LPR was directly correlated with the amount of HIV-1 that was obtained during plasmapheresis and that was used for pulsing DCs ($r = 0.55$; $P = .05$). After stop 2, the increase in LPR in the responders was not maintained. No change in LPR was observed in the control patients.

$CD4^+$ and $CD8^+$ T cell counts did not change significantly during the series of immunizations or after stop 2 (data not shown). However, at week 0, the responders had a higher CD4/CD8 index ($P = .001$), a higher naive $CD4^+$ T cell count ($P = .001$), and a lower memory $CD4^+$ T cell count ($P = .001$) than did the nonresponders. There were no differences at week 0 in the counts of other lymphocyte subsets or in HIV-1–specific immune responses between the responders and the nonresponders. At baseline (before receipt of any HAART), at week 30, and after stop 2, there were no differences in any immunologic parameters between the responders and the nonresponders.

Discussion. In the present study, we found that a vaccine comprising autologous MD-DCs pulsed ex vivo with heat-inactivated autologous HIV-1 was feasible, safe, and well tolerated

and elicited weak Th1 and HIV-1–specific $CD8^+$ T cell responses that were associated with a partial and transient control of viral replication. It could be argued that this vaccine did not elicit specific anti–HIV-1 immune responses at all; however, some data argue against this conclusion. First, we observed a very clear and consistent decrease in HIV-1–specific $CD8^+$ T cell responses during the series of immunizations, indicating that the patients were developing immunity. In fact, this decrease was not observed after the mock immunization at week 0. The reasons for this decrease in the number of circulating HIV-1–specific $CD8^+$ T cells are unclear, but others have reported similar results with respect to the immunization of patients with metastatic melanoma [10]. It has been speculated that this phenomenon might involve increased $CD4^+CD25^+$ regulatory T cell activity, the induction of HIV-1–specific $CD8^+$ regulatory T cells, increased apoptosis of activated $CD8^+$ T cells, or the trafficking of sensitized $CD8^+$ reactive T cells out of the peripheral blood [10, 11]. This last explanation is supported by the present findings—we observed that the increase in the number of total intrafollicular CTLs in lymphoid tissue and the decrease in the magnitude of the total $CD8^+$ T cell responses after immunization was correlated with the lengthening of the doubling time of PVL rebound from stop 1 to stop 2.

Second, virological response was associated with a weak but statistically significant increase in HIV-1–specific $CD4^+$ LPR. This increase in LPR was correlated with the amount of HIV-1 that was obtained during plasmapheresis and that was used for pulsing DCs, suggesting an antigen dose–related response. Therefore, we hypothesize that this induced helper response could permit $CD8^+$ T cells to recover the ability to proliferate [12] and could promote the differentiation of $CD8^+$ T cells into cytotoxic effectors [13] that migrate to lymphoid tissue at sites of HIV-1 replication and cell death [14], allowing partial control of viral replication in lymphoid tissue.

Although we found that DC vaccine did elicit cellular immune responses against HIV-1—even if weak and transient—the results of the present study are quite disappointing in terms of immunological and virological responses. It is unclear whether our findings resulted from DC dysfunction due to HIV-1 infection [15] or to technical aspects of the preparation of the DCs; these explanations are unlikely, however, because MD-DCs pulsed with a heat-inactivated whole laboratory strain of HIV-1 or recombinant HIV-1 proteins were able to induce the activation of autologous $CD4^+$ and $CD8^+$ T cells in vitro (data not shown). Other potential explanations could be found via a comparison of the present study with another recent study, one that was conducted in a population of HAART-naive patients with chronic HIV-1 infection and that included a schedule of therapeutic immunizations very similar to ours and autologous DCs pulsed with whole aldrithiol-2 (AT-2)–inactivated virus [5]. This study found that, after administration of 3 immunizations, PVL decreased by >90% for at least 1 year in 8 of 18 patients. This decrease in PVL was associated with strong and sustained HIV-1–specific cellular responses. The most important differences between the 2 protocols were as follows: (1) to pulse DCs, Lu et al. used a quantity of simian immunodeficiency virus that was 1000-fold higher than the quantity of HIV-1 we used; (2) Lu et al. inactivated virus with AT-2, whereas we inactivated virus with heat; and (3) Lu et al. obtained virus by culture, whereas we obtained virus by plasmapheresis. Whether these marked differences are relevant with respect to virological and immunological outcome should be answered in future trials.

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